AMENDMENTS TO THE SPECIFICATION

In the specification, please discount all amendments attempted in the response dated October 09, 2006, which amendments do not accurately match the pages of the present specification.

In the specification, at page 6, lines 20-29, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

In addition to the new immunization and screening techniques provided herein, antibodies that bind to aminophospholipids and anionic phospholipids and have a number of advantageous properties can now be identified by competition and/or functional assays using the monoclonal antibodies 1B9, 1B12, 3B10, 2G7, 7C5, 9D2 or 3G4. Currently, the 1B12, 3B10, 9D2 and 3G4 antibodies are preferred~~, as these antibodies do not require serum for phospholipid binding~~. The monoclonal antibodies 9D2 and 3G4 are more preferred, with monoclonal antibody 3G4 (ATCC 4545) currently being the most preferred. To identify additional antibodies that compete with any of the foregoing antibodies, preferably 3G4, the preferred assays are currently competition assays based upon an ELISA, a number of which are described herein, and working examples of which are disclosed.

In the specification, at page 9, lines 8-17, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

In certain aspects, the antibodies will effectively compete with the monoclonal antibody 1B9, 1B12, 3B10, 2G7, 7C5, 9D2 or 3G4, preferably with 9D2 or 3G4, and most preferably with 3G4 (ATCC 4545), for binding to an aminophospholipid or anionic phospholipid, preferably PS, or will have the aminophospholipid or anionic phospholipid binding profile of the monoclonal antibody 1B9, 1B12, 3B10, 2G7, 7C5, 9D2 or 3G4, preferably of 9D2 or 3G4, and most preferably of 3G4, as set forth in Table 4; ~~and will not be serum-dependent, *i.e.*, will not require serum to bind to the aminophospholipid or anionic phospholipid;~~ not be

derived from a patient with a disease, and will not significantly inhibit coagulation reactions *in vitro*, cause significant thrombosis *in vivo* or have lupus anticoagulant activities.

In the specification, at page 22, lines 14-17, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

All selection criteria~~, as used herein,~~ are preferably conducted in the absence of serum, to avoid the drawbacks with generating antibodies that could mimic the pathological antibodies of patients, which bind to aminophospholipids or anionic phospholipids in conjunction with proteins.

In the specification, at page 64, lines 29-35, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

~~The~~ Certain of the antibodies of the present invention have been particularly selected on the basis of not binding to aminophospholipids and anionic phospholipids in combination with protein cofactors, but rather are "true" anti-phospholipid antibodies. ~~As such, the~~ The antibodies of the invention do not ~~bind or~~ displace the protein cofactors from the phospholipids and are therefore safe for administration. Indeed, mice treated with the antibodies of the invention at high doses for prolonged periods showed no changes in coagulation capability, yet mice respond with APS when injected with anticardiolipin or lupus anticoagulant antibodies.

In the specification, at page 65, lines 21-28, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

In order to generate antibodies to aminophospholipids and anionic phospholipids with advantageous properties and/or reduced or essentially no side effects, the present invention provides preferred immunization and screening methods. Other immunization techniques and antibodies have been reported in the literature (Umeda *et al.*, 1989; Igarashi *et al.*, 1991; Rote *et al.*, 1993), including those with reported specificity for the type of fatty acid chains involved (Levy *et al.*, 1990; Qamar *et al.*, 1990). However, the present immunization techniques~~, and~~

~~particularly the selection of antibodies that are not serum dependent, provides~~ provide particular benefits.

In the specification, at page 66, lines 4-8, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The antibodies of the invention also have the advantage of recognizing all or most anionic phospholipids, which can provide more targets for binding. Therefore, the second generation antibodies of the invention can be defined as having substantially the same, or the same, phospholipid specificity as the 9D2 or 3G4 (ATCC 4545) antibodies, as disclosed herein in Table 4~~, and as not being serum dependent~~.

In the specification, from page 66, line 34 to page 67, line 4, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The antibodies of the present invention can also be characterized by their affinity. Prior to the invention, the antibodies in the literature had relatively weak affinity (where reported). In certain embodiments, the second generation antibodies of the invention are therefore defined as those that have an affinity for PS of at least equal to the affinity of the 9D2 or 3G4 (ATCC 4545) antibodies for PS, in particular, the affinity when measured in an ELISA as described herein, as disclosed in Table 3~~, and as not being serum dependent~~.

In the specification, at page 67, lines 6-13, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

More preferably, the second generation antibodies of the invention are defined as those having an affinity for PS of at least equal to the affinity of the 9D2 or 3G4 (ATCC 4545) antibodies for PS, as disclosed in Table 3, and as having substantially the same, or the same, phospholipid specificity as the 9D2 or 3G4 (ATCC 4545) antibodies, as disclosed in Table 4~~, and as not being serum dependent~~. Most preferably, the second generation antibodies are those having an affinity for PS of at least equal to the affinity of the 3G4 (ATCC 4545) antibody for

PS, as disclosed in Table 3, and as having the same phospholipid specificity as the 3G4 (ATCC 4545) antibody, as disclosed in Table 4~~, and as not being serum dependent~~.

In the specification, at page 68, lines 26-32, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Further aspects of the invention concern at least one CDR that has a CDR amino acid sequence encompassed by the variable region amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or a variant or mutagenized form thereof. Other aspects of the invention concern a CDR, antibody, or antigen binding region thereof, which binds to at least a first aminophospholipid or anionic phospholipid, preferably PS, and which comprises at least one CDR with a CDR amino acid sequence encompassed by the variable region amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or a variant or mutagenized form thereof, wherein such a variant or mutagenized form maintains binding to the aminophospholipid or anionic phospholipid, preferably PS.

In the specification, at page 71, lines 23-31, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

Further aspects of the invention concern an isolated polynucleotide that contains a nucleotide sequence that encodes at least one CDR that has a CDR amino acid sequence encompassed by the variable region amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or a variant or mutagenized form thereof. Other aspects of the invention concern an isolated polynucleotide that contains a nucleotide sequence that encodes a CDR, antibody, or antigen binding region thereof, which binds to at least a first aminophospholipid or anionic phospholipid, preferably PS, and which comprises at least one CDR with a CDR amino acid sequence encompassed by the variable region amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4, or a variant or mutagenized form thereof, wherein such a variant or mutagenized form maintains binding to the aminophospholipid or anionic phospholipid, preferably PS.

In the specification, at page 73, lines 1-10, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

In particular embodiments, the invention concerns isolated coding segments or isolated gene portions and recombinant vectors incorporating DNA sequences that encode ~~CDR~~ variable regions of anti-anionic phospholipid or anti-aminophospholipid antibody heavy and light chains, such as 9D2 and 3G4, and preferably 3G4, heavy and light chains, that comprise at least a first sequence region that includes an amino acid sequence region of at least about 75%, more preferably, at least about 80%, more preferably, at least about 85%, more preferably, at least about 90%, 91%, 92%, 93%, 94%, and most preferably, at least about 95%, 96%, 97%, 98% or 99% or so amino acid sequence identity to the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4; wherein said ~~CDR~~ variable regions at least substantially maintain the biological properties of the ~~CDR~~ variable regions of amino acid sequences SEQ ID NO:2 or SEQ ID ~~NO:2~~ NO:4.

In the specification, from page 180, line 29 to page 181, line 4, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The pathogenic anti-phospholipid antibodies that circulate in patients with antiphospholipid syndrome are believed to bind to PS, PE and other phospholipids in combination with proteins, such as β_2-glycoprotein I, prothrombin, kininogens, prekallikrein and factor XI (Rote, 1996; Sugi and McIntyre, 1995; 1996a; 1996b). β_2-glycoprotein I and prothrombin bound to PS are reported to be the primary antigens for anti-cardiolipin antibodies and lupus antibodies, respectively. ~~The~~ Certain of the antibodies of the present invention have been particularly selected on the basis of not binding to aminophospholipids and anionic phospholipids only in the presence of serum proteins. ~~Therefore, by binding to the phospholipid component, the~~ The antibodies of the invention are contemplated for use in antagonizing or competing with the pathogenic antibodies in such patients, thus displacing the pathogenic antibodies from their phospholipid-protein targets in the body.

In the specification, at page 216, lines 5-11, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

An important aspect of the inventors' technique to prepare monoclonal antibodies useful in tumor treatment is the selection strategy, which involves screening to select antibodies that bind to aminophospholipids or anionic phospholipids, but not to neutral phospholipids. ~~Another important~~ A certain aspect is to select antibodies that bind to PS-coated plates as strongly in the presence of serum as in the absence of serum. ~~This is carried out to exclude antibodies that recognize complexes of PS and serum proteins, which are believed to cause or contribute to anti-phospholipid syndrome.~~

In the specification, at page 218, in Table 2, please delete the existing table and replace the deleted table with the following table after making the following changes:

TABLE 2

Isotype and Serum-Dependence of Anti-PS Antibodies

Name	Origin	Species/Isotype	Serum-dependence
3SB	Rote *et al.*, 1993	Mouse IgM kappa	None
D11	N. Rote	Mouse IgM kappa	
BA3	Rote *et al.*, 1993	Mouse IgM kappa	
9D2	This study	Rat IgM kappa	None
1B12	This study	Mouse IgG_1 kappa	
3G4	This study	Mouse IgG_3 kappa	~~None~~ Yes
1B9	This study	Mouse IgG_1 kappa	Absolute
3B10	This study	Mouse IgG_3 kappa	~~None~~ Yes
2G7	This study	Mouse IgG_1 kappa	Absolute
7C5	This study	Mouse IgG_1 kappa	Absolute

In the specification, at page 220, lines 16-22, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The 1B9, 2G7 and 7C5 antibodies behave essentially the same. These antibodies recognize only PS and require serum or serum proteins for binding to PS. The binding of 1B9, 2G7 and 7C5 to various phospholipids was assayed only in the presence of 10% bovine serum~~, whereas binding of the other antibodies was tested either in the absence or in the presence of serum~~. For ~~antibodies other than 1B9, 2G7 and 7C5~~ the 9D2 antibody, the presence of serum does not change preference in binding to a particular phospholipid. ~~This latter group, including 3G4, 3B10 and 9D2, have the preferred property of binding~~ The 9D2 antibody binds to PS in the absence of serum.

In the specification, at page 274, lines 6-11, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The ~~epitope recognized by 3G4 appears to lie within the~~ phosphoglycerol core of the anionic phospholipids~~, which~~ is the same in phospholipids from all mammalian species. The 3G4 antibody ~~thus~~ reacts with both mouse and human phospholipids, which is important for pre-clinical and clinical development. 3G4 is more specific for anionic phospholipids than the natural ligand, annexin V. Unlike 3G4, annexin V also binds strongly to neutral phospholipids in physiological concentrations of Ca^{2+}.

In the specification, at page 277, lines 15-20, please delete the following paragraph:

An important aspect of the 3G4, 9D2 and like antibodies stems from the inventors' realization that desirable antibodies should preferably be selected using a screen to identify antibodies that bind to PS-coated plates as strongly in the presence of serum as in the absence of serum. This new development provides the ability to identify and exclude antibodies that recognize complexes of PS and serum proteins, as such complexes are believed to be the cause of, or an important factor in, anti-phospholipid syndrome and associated pathologies.

In the specification, at page 280, lines 2-5, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The original sequences of the antibody variable regions were obtained by RACE from the hybridoma that produces the 3G4 antibody and the sequences verified. The nucleic acid and amino acid sequences of the variable region of the heavy chain (Vh) of the 3G4 antibody, encompassing CDR1-3, are represented by SEQ ID NO:1 and SEQ ID NO:2, respectively.

In the specification, at page 280, lines 22-31, please delete the existing paragraph and replace with the following paragraph after implementing the following changes:

The nucleic acid and amino acid sequences of the variable region of the light chain (Vκ) of the 3G4 antibody, encompassing CDR1-3, are represented by SEQ ID NO:3 and SEQ ID NO:4, respectively. SEQ ID NO:3 and SEQ ID NO:4 again include part of the mouse leader sequence and constant chain sequences, as shown in FIG. 18B. The leader sequence is amino acids 1 through 22 of SEQ ID NO:4, and the mature protein begins as shown by the arrow in FIG. 18B. Sufficient complementarity determining region sequence information is included by the sequence of the mature protein up to the sequence portion concluding TVF, after which the amino acids are not essential for antigen binding. As such, the BbsI site in the nucleic acid sequence can be used as a convenient site to prepare a functional mouse variable region, *e.g.*, for use in grafting onto a human constant region (FIG. 18B).